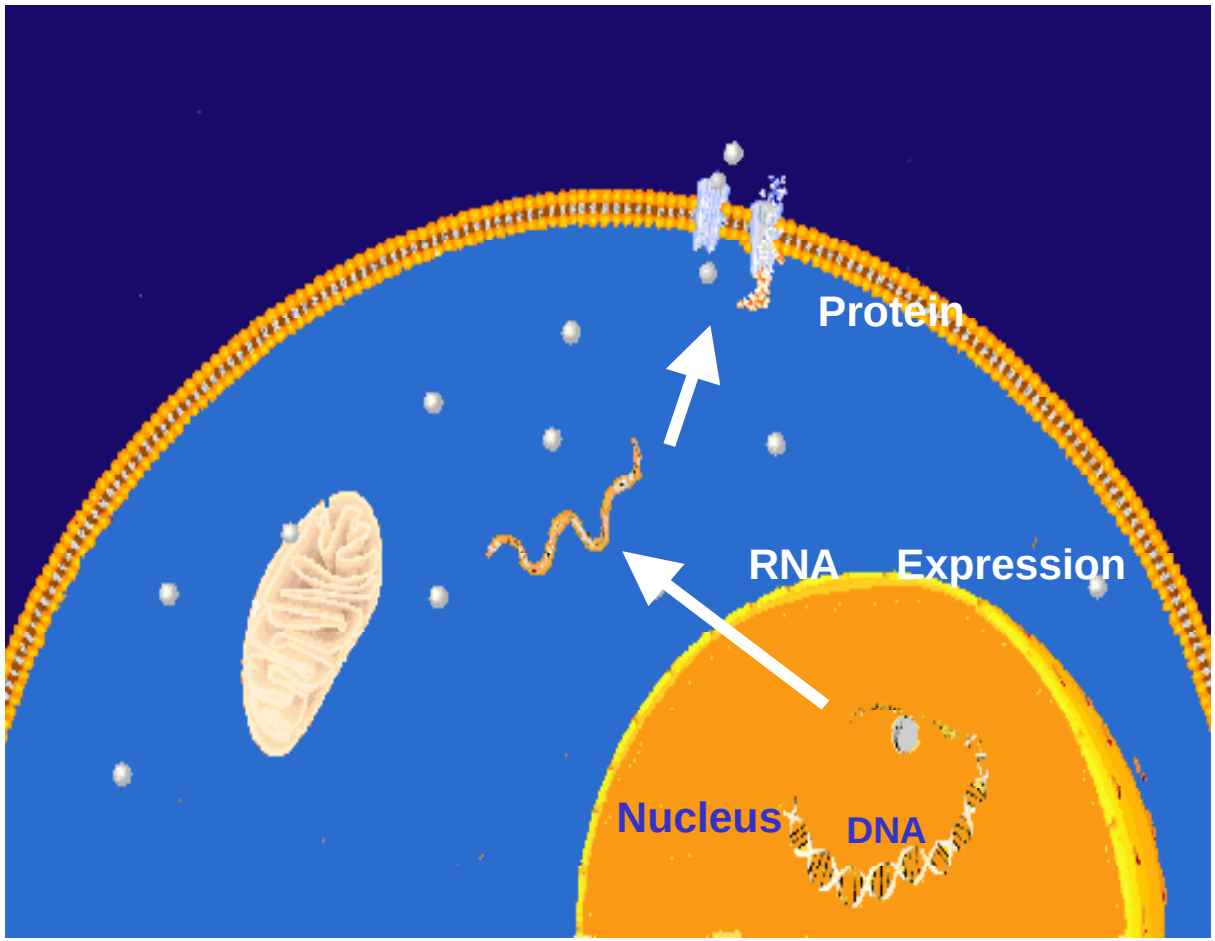
Gene Expression:

DNA —Transcription→ **mRNA** —Translation→ **Protein**

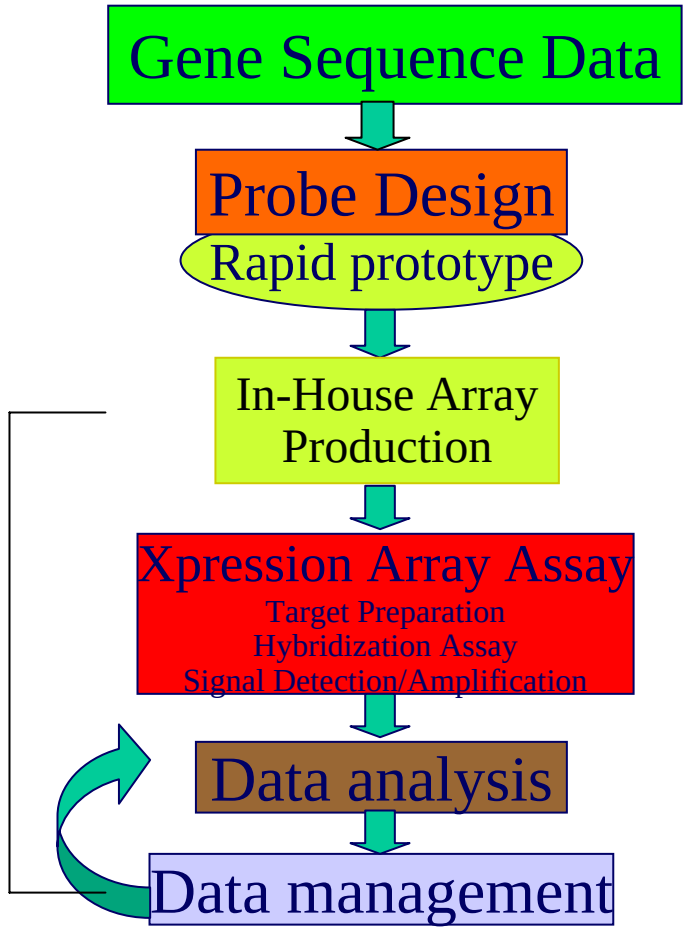


Expression Assay Process Flow

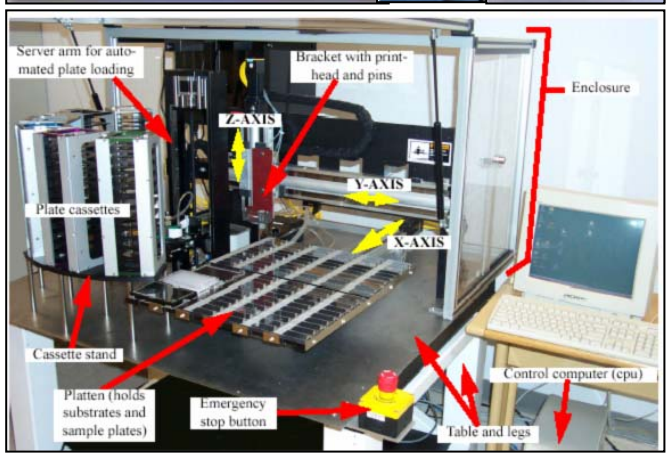
Public/Private DB

OligoLibrary Vendor

KCC/TJU
Microarray Facility

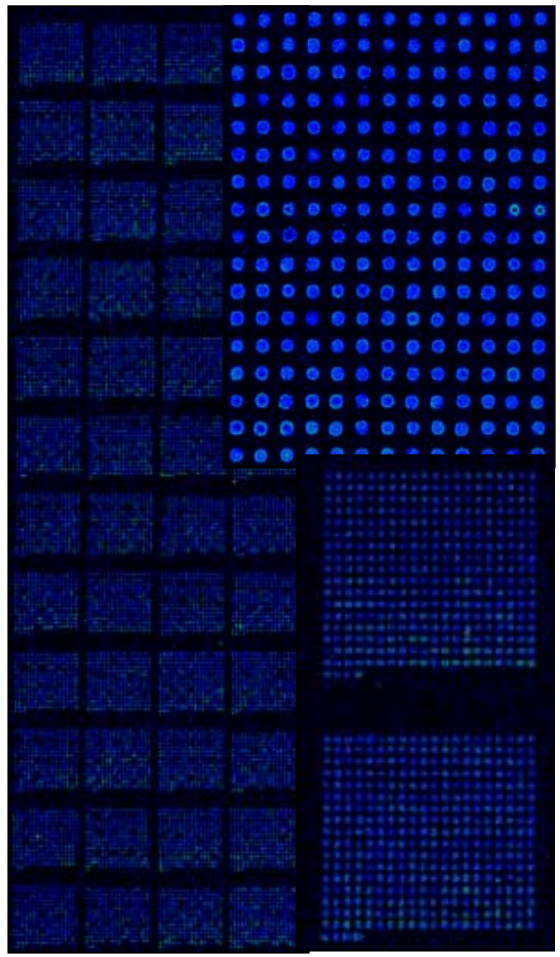


KCC-TJU Bioarray Manufacturing



- **Precise-- contact printing**
- **aspiration volume**
  - ✓ **325 pL volume, ~20μM**
- **25,000 elements per 1”x3” format**
  - ✓ **100 μm spots**
  - ✓ **200 μm pitch**
  - ✓ **~2200 probes/cm²**
- **Rapid and flexible**
- **Fluorescent dye codispensed with oligo for printing QC.**
- **Oligo QC**

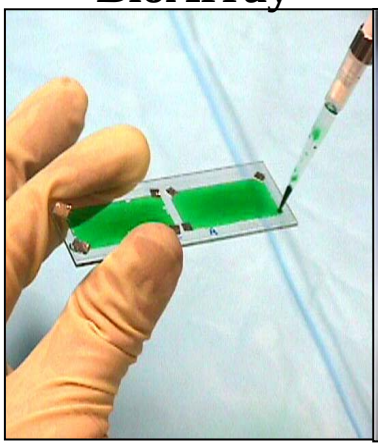
✓ **Purity Confirmation (mass spec)**



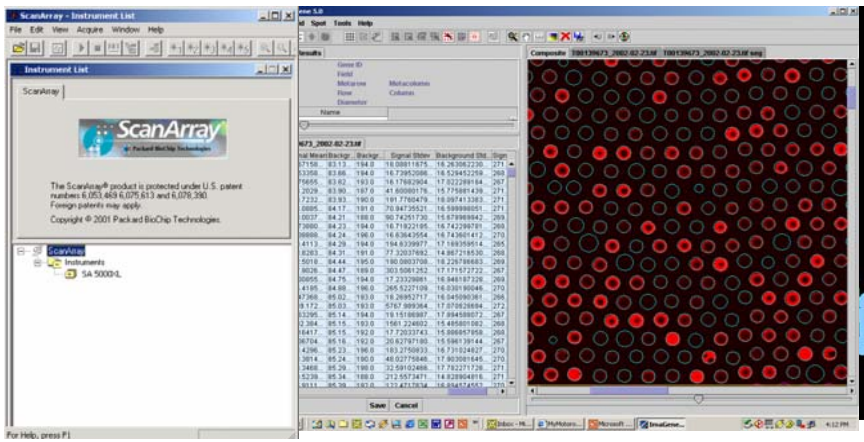
KCC Microarray Facility for Genomic Research

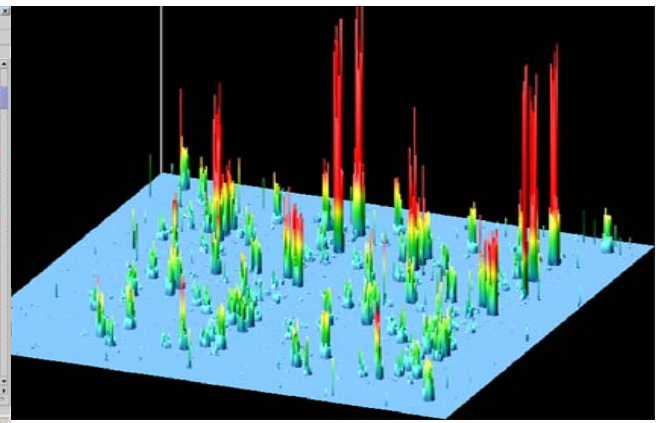
Array, Instrumentation and Software

BioArray | **Hybridization** | **Signal Detection** | **Scanning**



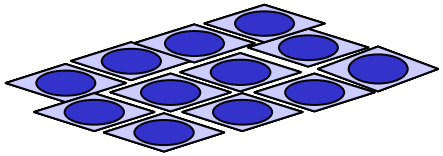
ScanArray software | **QuantArray software**



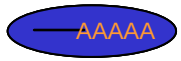


Gene Expression Technologies

----Targets Preparation



Cultured Cells



Lyse

AAAAA

Reverse Transcription

mRNA

cDNA

AAAAA

TTTTT-T7

Second -Strand Synthesis

ds-cDNA

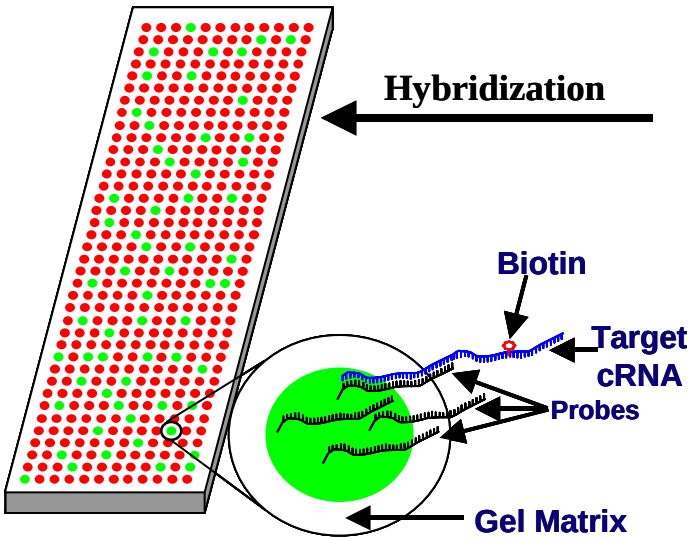
TTTTT-T7

*NTP Label Incorporation

IVT

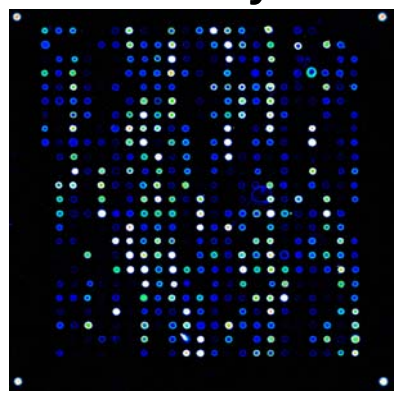
* * * * * * * * * * * * * * * TTTTT

Hybridization



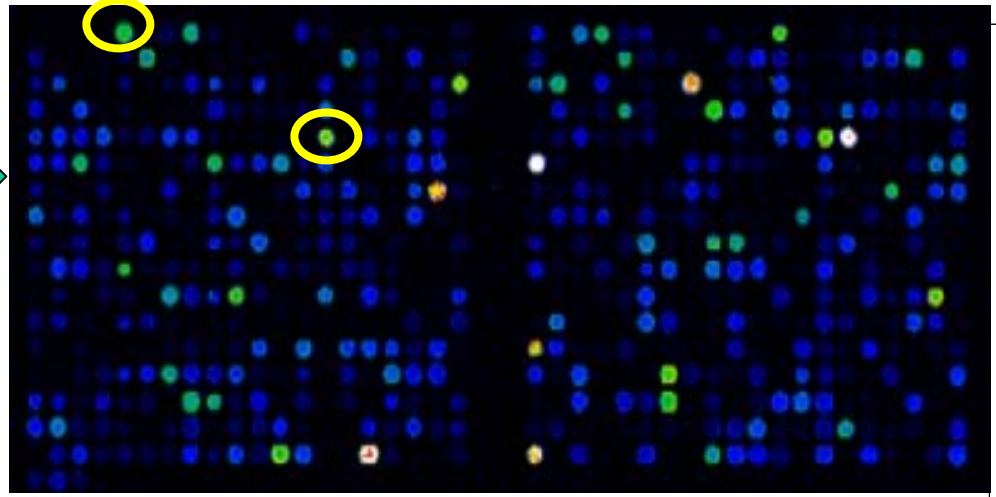
Direct Detection or Signal Amplification

Bioarrays

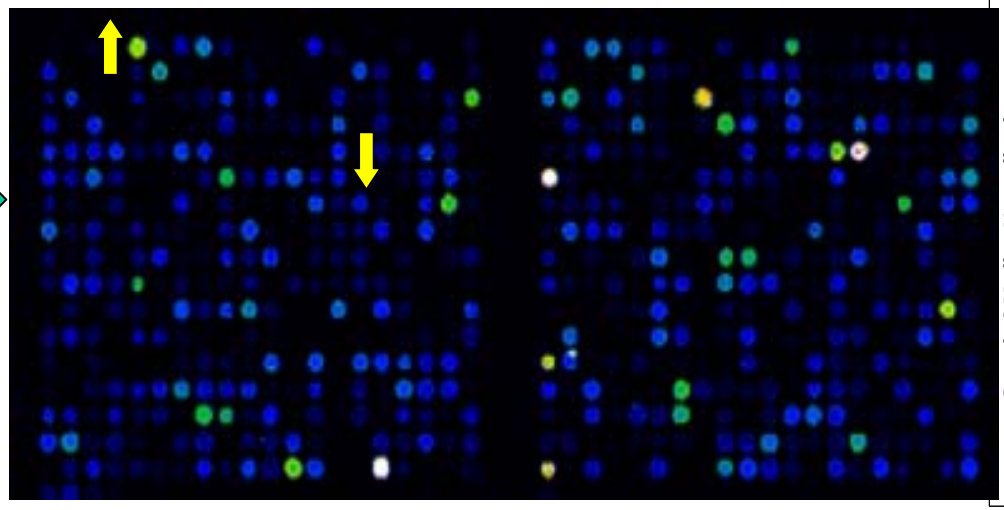


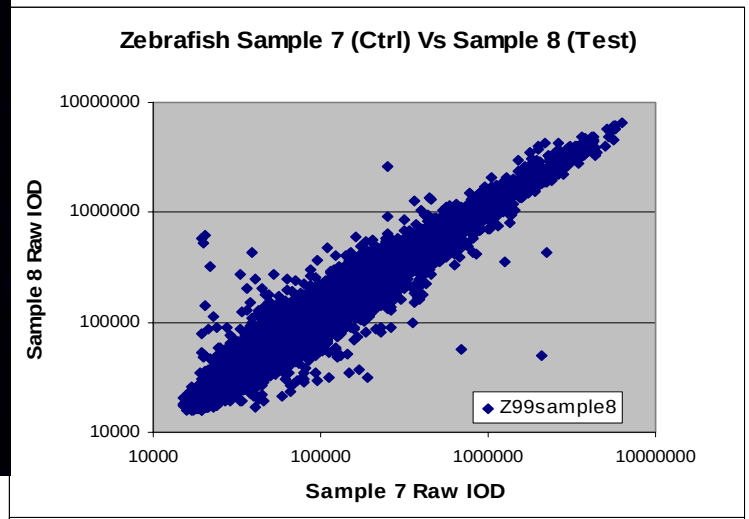
Biological Differential Gene Expression Between WT and Mutant on Zebrafish 17K Expression Microarray

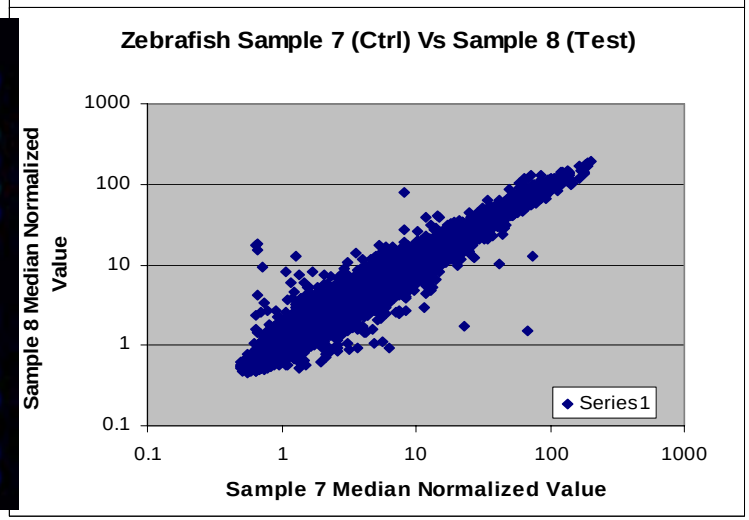
WT



Mutant





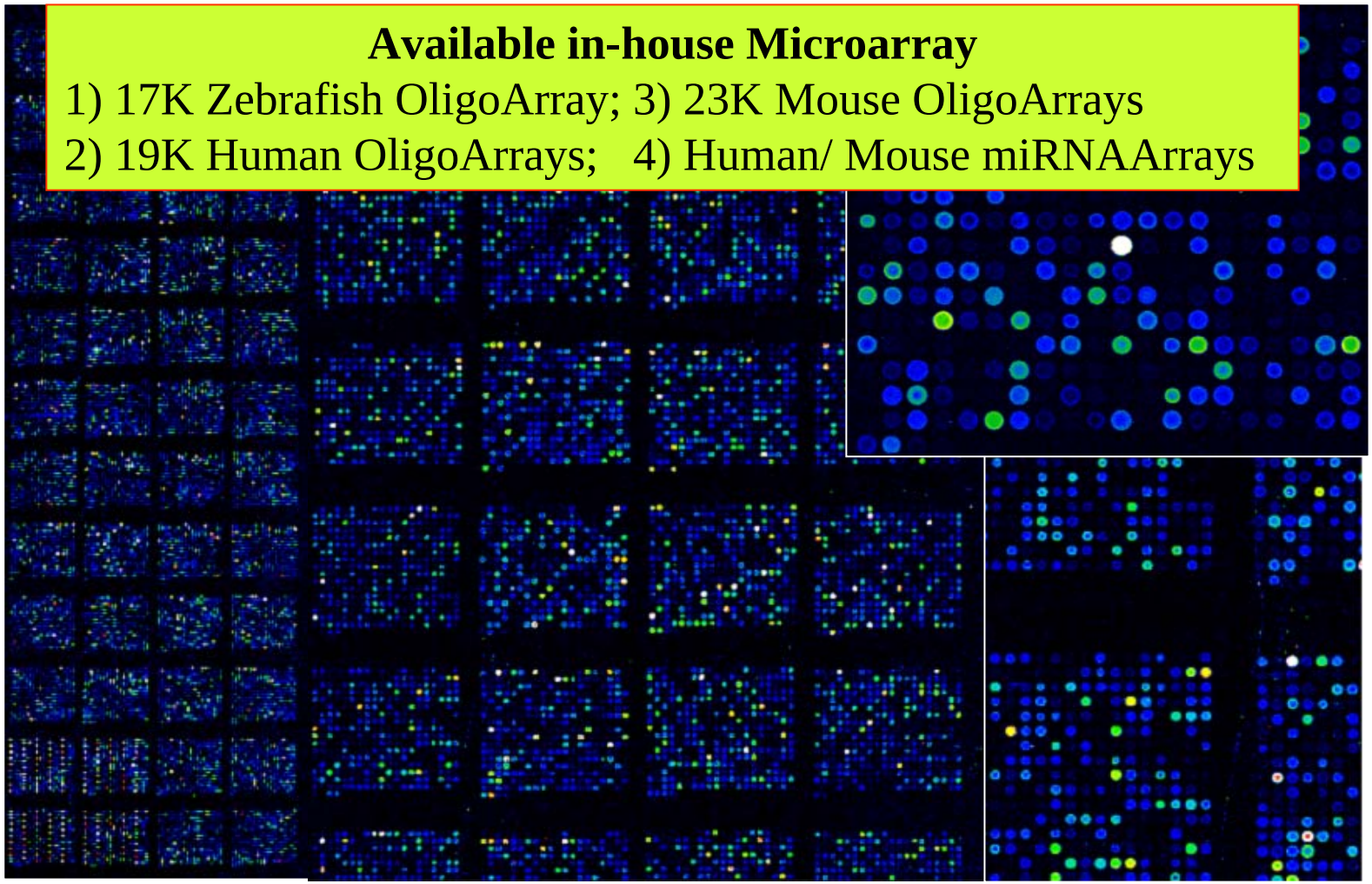


Gene expression array for genome-wide expression monitoring

Available in-house Microarray

1) 17K Zebrafish OligoArray; 3) 23K Mouse OligoArrays

2) 19K Human OligoArrays; 4) Human/ Mouse miRNAArrays



Updated Gene Expression Profiling Services

Done

1) Zebrafish Microarrays

450 biological samples from 40 labs. in US, UK, Germany, Singapore, Hong-Kong have been processed

2) Human Microarrays

100 Samples from six labs at TJU have been processed.

3) Mouse Microarrays

120 Samples from Seven labs at TJU have been processed.

4) Rat Microarray

12 Samples from two labs at TJU have been processed.

5) Human / Mouse miRNA Microarray

100 Human CLL, Mouse and Rat Samples have been Processed

Zebrafish Expression Microarray Users

450 biological samples from 40 Research Laboratories

Dr. Sharon Amacher	University California	Mesoderm patterning and segmentation
Dr. Becker Catherina	University Hamburg	Identify gene expressed during neuronal and retinal regeneration
Dr. Carvan Micheal	University Wiscosin	Effects of Toxic Chemicals on developmental processes
Dr. James Casey	Cornell University	Expression of genes activated in oncogenic pathway
Dr. Keith Cheng	Penn State University	Mutants and morphants in the area of genomic instability, cell differentiation and cancer
Dr. Xavier Cousin	INRA	Effects of drugs on the process of muscule differentiation
Dr. Clark Ted	Cornell University	Infectious disease immunology in human and animals
Dr. Kristin Edepli	MIT	Analyze various mutants
Dr. Stephen Ekker	University Minnesota	Analyze a series of morphants and mutants coming from screens
Dr. Shannon Fisher	Johns Hopkins University	Gene misregulated in the mutant osteoblasts during embryogenesis and fin regeneration
Dr. Rhowen Ge	National University Singapore	Analyze VEGF regulated genes during early development
Dr. Giancarlo Ghiselli	Thomas Jefferson University	Identify gene differentially regulated by proteoglycans during development
Dr. Daniel Goldman	University Micheagan	Identify genes induced in the retina during optic nerve regeneration
Dr. Marnie Halpern	Carnegie Institution Washington	Identify gene differentially regulated expressed on the left and right sides of zebrafish forebrain
Dr. TinChung Leung	Geisinger Health System	Developmental roles of Orphan G protein Coupled receptors
Dr. Corinne Houart	King's college London	Early forebrain patterning
Dr. John Mably	Harvard Medical School	Expression profiling of zebrafish mutant lines
Dr. Micheal Pack	University Pennsylvania	Gene expression patterns in gut mutants to define novel patterns of organogenesis
Dr. David Parichy	University Texase at Austin	Pigment patterns and developmental genetics of metamorphosis
Dr. Tracie Payne-Ferreira	The Forsyth Institute	Downregulation of genes in response to decreased receptor mitf in mutants
Dr. Erez Raz	Max-Planck Institute	Germ cell migration
Dr. Sandor Shapiro	Jefferson Medical College	The role of filamins embryologic development
Dr. Wiliam Voigt	St. Louis University	Role of extracellular ATP in the developing mature nervous system
Dr. Andrew Waskiewicz	University Alberta	Gene downstream of homeodomain Transcription factors in the hindbrain
Dr. Eric Weinberg	University Pennsylvania	analyze role of B-catenin in organizer formation and anteroposterior patterning
Dr. Eric Wickstrom	Thomas Jefferson University	Regulation of human oncogene orthologues
Dr. David Hyde	University Notre Dame	
Dr. Elwood Linney	Duke University	
Dr. Daniel Hart	U. Mass. Medical School	
Dr. Mark Mellon	University of Maine	
Dr. David Raible	University Washington	
Dr. Steve Farber	Thomas Jefferson University	
Dr. William Jeffery	University Maryland	
Dr. Suresh Jesusthasan	National University of Singapore	
Dr. TaoZhong	Vanderbilt University	
Dr. Catherine Verfaille	University Minnesota	
Dr. Vicky Prince	University Chicago	
Dr. Adam Dicker	Thomas Jefferson University	
Dr. Weiming Li	University Micheagan	
Dr. Kathleen Whitlock	Cornell University	

caBIG Integrated Cancer Research Workspace Contribution by KCC

Collection of all data pertaining to microarray experiments and distribution of experimental results:

The Kimmel Cancer Center is pioneering the use of a number of exclusive in-house built chips (eg. microRNA chip). It is well known that experimental design and conditions greatly influence results of microarray. The influence of all these factors will be studied as the database reaches a critical mass.

Based on applications KCC developed for the world-wide zebra fish consortium, we will provide secure, web-based access to the large files of microarray results generated at a parent center to community of users at institutions world-wide. Project also includes applications for microarray facility user registration and service requests (including experiment description based on MIAME standard).

Software Architecture

System design:

Web-based databases for acquiring and retrieving user and experiment information, and controlling access to file directories containing result files to be downloaded from web.

Component details:

Applications to establish user accounts and result folders, accession requests, authenticate users, distribute results.

Relevant standards:

FTP, HTML, PostgreSQL, PHP, Apache, MIAME

MicroArray Facility: Request User Account -- step - Microsoft Internet Explorer

File Edit View Favorites Tools Help

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Address http://www.kimmelcancercenter.org/Science/SharedFacilities/MicroArray/RequestUserAccount-1.asp

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Philadelphia, Pennsylvania
(888) 955-1212

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MICROARRAY FACILITY: Request for User Account

NOTE: All fields MUST be completed.

User's Last Name	
User's First Name	
Thomas Jefferson University Faculty or Staff ?	
P. I.	
Location	
Phone number	
Charge Code	
Email Address	
continue	reset

MicroArray Facility Sign-In - Microsoft Internet Explorer

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Address http://www.kimmelcancercenter.org/Science/SharedFacilities/MicroArray/default.htm

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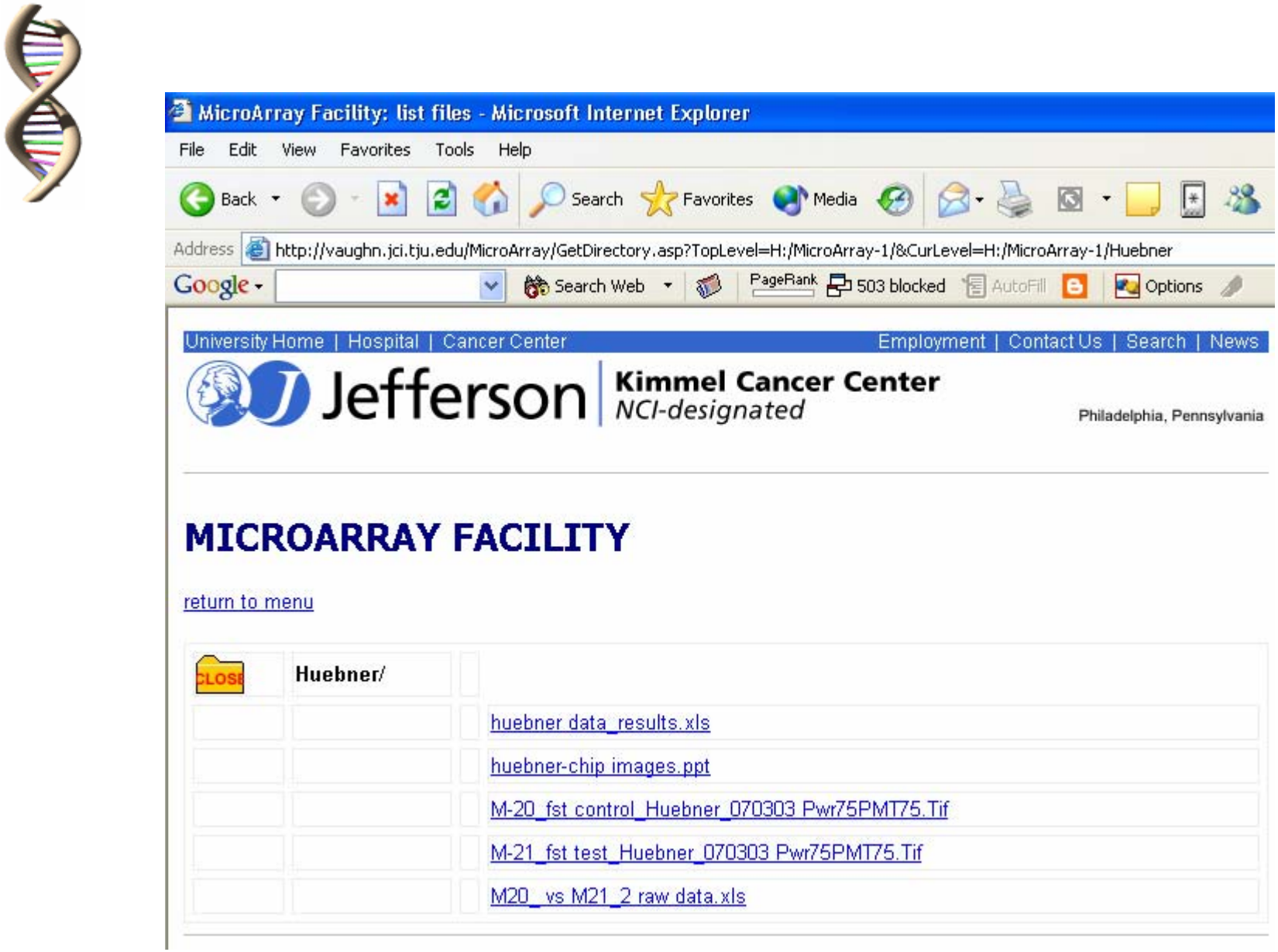
MICROARRAY FACILITY

To access the databases, you must first enter your username and password:

UserName

Password

Continue


MicroArray Facility: list files - Microsoft Internet Explorer
File Edit View Favorites Tools Help
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huebner-chip images.ppt
M-20_fst control_Huebner_070303 Pwr75PMT75.Tif
M-21_fst test_Huebner_070303 Pwr75PMT75.Tif
M20_ vs M21_2 raw data.xls

MicroArray Facility: list files - Microsoft Internet Explorer

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Address http://vaughn.jci.tju.edu/MicroArray/GetDirectory.asp?TopLevel=H:/MicroArray-1//&CurLevel=H:/MicroArray-1//&staff=&folder=

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- H:/MicroArray-1/antisense Cy3 oligo tesing/
- H:/MicroArray-1/Arthur Buchberg/
- H:/MicroArray-1/Bruno Calabretta/
- H:/MicroArray-1/Compugen/
- H:/MicroArray-1/DOE testing/
- H:/MicroArray-1/Emad Alnemri/
- H:/MicroArray-1/Farber/
- H:/MicroArray-1/Gcaline/
- H:/MicroArray-1/Genisphere/
- H:/MicroArray-1/Ghiselli/
- H:/MicroArray-1/Huebner/
- H:/MicroArray-1/Human/
- H:/MicroArray-1/JW/

MicroArray Submission Form - Microsoft Internet Explorer

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Address http://www4.kimmelcancercenter.org/microarray/microarray_submission.php Go Links Norton AntiVirus

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menu | sign-in | **Microarray Facility**

SERVICE REQUEST FORM

Investigator Information

Name

Address

Affiliation

Email

Phone Number

Date

Type of Experiment

- Normal vs. Diseased ☐
- Treated vs Untreated ☐
- Time Course ☐
- Dose Response ☐
- Effect of Gene KO ☐
- Effect of Gene KI ☐

Experimental Factors (parameters, conditions tested)

- Time ☐
- Dose ☐
- Genetic Variation ☐
- If Common Reference is used for all hybridization?? ☐
- If Replicates Done ☐
- Dye Swap Used ☐

Type of Replicates

Other Details

Experiment Description, Goals

Links, URL

Samples Used

Done Internet

start Calendar -... Microsoft ... 3 Intern... Kimmel Ca... mercury - ... Microsoft ... Adobe Ph... 2 pgAdm... 4:00 PM

Samples Used

Bio-Source Properties

Organism (NCBI) Taxonomy

Contact Details

Description of Sample

Sex of Organism female male

Age of Organism

Development Stage

Organism part (tissue)

Cell type

Animal/Plant Strain

Genetic Variation

Individual Genetic Characteristics

State Disease Normal

Additional Clinical Information (Links)

Biomaterial Manipulations

Growth Conditions

In vivo Treatments

In vitro Treatments

Treatment Type

Compound

Separation Technique

Hybridization Extract Preparation Protocol

Extraction Method

Total RNA

mRNA

Genomic DNA

Labeling Protocol

Amount of Nucleic Acid Labeled

Label Used

Array Batch

Array Serial Number

Hybridization Protocol

Concentration of Solutes

Blocking Agent

Wash Procedure

Quantity of Labeled Targetget

Time

Concentration

Volume

Temperature

Measurement of Data and Specifications of Data Processing

Raw Data Description

Scanning Protocol

Scanning Hardware

Scanning Software

Scanned Images At:

Scan Parameters

Laser Power

Spatial Resolution

Pixel Space

PMT Voltage

Image Analysis and Quantitation

Image Analysis Software

Image Analysis Output At:

Normalized and Summarized Data - Gene Expression Data Matrix

Data Processing Protocol

Gene Expression Data Table(s)

Submit Reset

caBIG Integrated Cancer Research Workspace Contribution by KCC

12-month work plan:

Months **1-3**: complete enhancements currently underway to basics applications; draft administrative and user documentation.

Months **4-5**: validate documentation, software and security using the zebrafish community.

Months **6-8**: migrate software to and train personnel at caBIG adopter sites.

Months **9-12**: monitor use and provide updates as needed.